Association of genetic polymorphism of CYP2C19, P2Y12 and response to Clopidogrel in healthy participants.

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ABSTRACT

Clopidogrel, a Thienopyridine derivative, irreversibly inhibits platelet aggregation by antagonizing the action of adenosine diphosphate (ADP) on purinergic (P2Y12) receptor. It is used in acute coronary syndromes, ischemic stroke and also recently indicated for atrial fibrillation. Clopidogrel is a prodrug requiring biotransformation mainly by the hepatic Cytochrome P450 enzyme 2C19 (CYP2C19) to generate an active metabolite. Studies done in the west have shown a large variability in the clopidogrel response. It has been reported that the variant forms of CYP2C19 gene are associated with poor responsiveness to the drug. There is no data available in the Indian population and hence the present study was envisaged with the objective of evaluating the association of polymorphisms of CYP2C19 and P2Y12 with inhibition of platelet aggregation (IPA) to a single oral dose of 300 mg Clopidogrel in adult Indian healthy participants. Healthy adults (n=25) of either sex were enrolled after obtaining written informed consent and administered single dose of 300 mg clopidogrel. Blood samples (9 ml) were collected for platelet aggregation tests at the baseline (pre-dose), 4 hours post-dose. Additionally, 5 ml blood was collected from each participant for genotyping. Baseline and four hours post-dose of platelet aggregation and genotype of CYP2C19 and P2Y12 were carried out using standardized laboratory methods. The difference in the maximal platelet aggregation (MPA) between baseline and four hours post-dose was considered as delta-MPA (DMPA) and percent change of MPA at four hours from baseline was considered as inhibition of platelet aggregation (IPA). Those with an IPA of < 30% were considered as poor responders. Inferential statistics was applied to find out significant difference of these parameters between various groups of genetic polymorphisms. Wide range of inter individual variations to Clopidogrel response was noted in the 25 healthy volunteers. Mean (SD) of MPA (%) at baseline and 4 hours post-dose were 75.8 (6.5) and 54 (17) respectively. Similarly, mean (SD) of DMPA (%) and IPA (%) were 21.8 (16) and 29 (21.5) respectively. A total of 13/25 (52%) were found to be poor responders to clopidogrel. A wild genotype (*1/*1) of CYP2C19 was observed in 15 (60%), 8 (32%) had *1/*2 and 2 (8%) had *2/*2 mutant genotypes. Although statistically not significant (p = 0.3624), a trend was observed in having decreased inhibition values (both MPA and IPA) as we proceed from wild genotype (*1/*1) to the mutant genotypes in this order: *1/*2 and *2/*2. Similarly, in P2Y12, a wild haplotype (H1/H1) was present in 20 (80%) and 5 (20%) individuals had H1/H2. No significant difference or trend was observed in DMPA or IPA values between H1 and H2 haplotypes of P2Y12. We found a trend of decrease in the inhibition of platelet aggregation with CYP2C19 genotypes and an increase in the same with the H2 haplotype of P2Y12 following clopidogrel in Indian healthy adults. Assessment of genetic polymorphisms of the same may aid in personalizing the therapy with clopidogrel.

KEYWORDS: Clopidogrel, CYP2C19, P2Y12, polymorphism.

INTRODUCTION

Coronary artery disease (CAD) is a condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardium. It typically occurs when there is an imbalance between myocardial oxygen supply and demand. It is the first

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among top 5 causes of deaths in Indian population. In 2000, there were an estimated 29.8 million people with CHD in India out of a total estimated population of 1.03 billion, or a nearly 3% overall prevalence (Gupta R et al., 2008). According to World Bank estimates, CVD had a 31% share in the total burden of disease in 2001 (Peters D et al., 2001). The most common cause of myocardial ischemia is atherosclerotic disease of an epicardial coronary artery or arteries sufficient to cause a regional reduction in myocardial blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery. Acute coronary syndrome is a spectrum of clinical conditions from unstable angina, non-ST-segment elevation myocardial infarction (MI), ST-elevation MI, and sudden death. Clinically, acute chest pain, typical in character, lasts more than 15 minutes. 'Typical' defined as retrosternal discomfort (heaviness), brought about or increased with exertion and reduced with rest or nitrates (WHO Collaborative programme, 2008-2009). Platelets play an essential role in the pathogenesis of acute coronary syndromes (ACS). Therefore an important part of the treatment of ACS, and of primary and secondary preventive measures in coronary heart disease, consists of antiplatelet treatment (Valentin Fuster, 1992). Across the spectrum of acute coronary syndrome and in patients undergoing percutaneous coronary interventions (PCI) with stenting, dual antiplatelet therapy with Aspirin (75-150mg) and Clopidogrel (300mg), a thienopyridine inhibitor of the platelet P2Y12 receptor, is the standard of care (Chris Terpening et al., 2009). Clopidogrel, [methyl (2S)-2-(2-chlorophenyl)-2-(6, 7-dihydro-4H-thieno [3, 2-c] pyridin-5-yl) acetate], is an oral, thienopyridine-class antiplatelet agent, that irreversibly inhibits platelet aggregation by selectively binding to adenylate cyclase-coupled ADP receptors on the platelet surface. It reduces the risk of thrombotic events in patients with a history of atherosclerotic diseases, such as stroke or myocardial infarction (Coukell AJ et al., 1997). Thienopyridines are inactive prodrugs and the active moiety, of their active metabolite is a reactive thiol derivative that targets P2Y12 on platelets. The active metabolite of clopidogrel binds specifically and irreversibly to the platelet P2Y12 purinergic receptor, inhibiting ADP-mediated platelet activation and aggregation. (Kim KA et al., 2008). The prodrug clopidogrel requires oxidation by the hepatic cytochrome P450 (CYP) system to generate active metabolites. CYP2C19 has an important function in the metabolism of clopidogrel to its active metabolite, however other members of the CYP family are also involved (e.g. CYP3A4, CYP3A5, CYP2B6 and CYP1A2) (Amber L. Beitelshesee et al., 2006). It was found that coexistence of the polymorphism of P2Y12 and the polymorphism of CYP2C19 was associated with persisting platelet activity in patients with ACS on clopidogrel treatment, which suggested that coexisting, rather than single, polymorphisms of different genes may be related to persisting platelet activation while on clopidogrel, which raises concern about harm in patients with ACS (Malek LA et al., 2008). Similar studies found that subjects with genotypes of CYP2C19*2 and CYP2C9 associated with reduced function had decreased exposure to the active metabolite and decreased pharmacodynamics response to clopidogrel. These subjects were also more prone to high-on clopidogrel platelet reactivity, which is associated with poor clinical outcome after coronary stent placement (Brandt JT et al., 2007). Similar results were obtained in studies with P2Y12 polymorphisms, which found that subjects undergoing clopidogrel treatment, who were carriers of the P2Y12 polymorphisms had a 4-fold higher risk to have an adverse neurological event than subjects carrying the wild-type genotype. Sequence variation of the P2Y12 was likely to alter affinity of platelets to ADP. The analyses suggest that genetic variation of the P2Y12 receptor gene was associated with higher numbers of cerebrovascular events in patients receiving clopidogrel therapy (Ziegler et al., 2005).

**Material and Methods**

**Ethics:**

The study was initiated after obtaining the approval from Institutional ethics committee of the institute. Only after written informed consent is obtained from all potential participants they will be recruited. A detailed explanation will be given to all the participating patients regarding the study and their contribution to it. The study was conducted according to ICMR guidelines for biomedical research in human subjects, 2006; schedule Y of Drugs and Cosmetic rules, 2005 and ICH-GCP guidelines, 1996. The study was registered with Clinical Trials Registry of India (CTR/2011/06/001795).

The Study was conducted at “Department of Clinical Pharmacology, Seth GS Medical College and King Edward Memorial Hospital, Acharya Donde Marg, Parel, Mumbai 40012 India”.

**Sample size estimation:**

No formal sample size calculations have been made for the study. It was expected that, this study provides background information which will form the basis for future studies.

**Study subjects:**

25 blood samples from healthy volunteers were obtained for the study. Participants were screened from the paramedical staff of Seth GSMMC & KEM, Hospital and past healthy volunteer pool. No formal advertisement were made.
Figure-1. Depicts the details of the study conducted.

**FULL DETAILS (Read-only)**

<table>
<thead>
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<td>14/03/2013</td>
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<tr>
<td>Post Graduate Thesis</td>
<td>No</td>
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<td>Type of Trial</td>
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<td>Type of Study</td>
<td>Drug</td>
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<tr>
<td>Study Design</td>
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<tr>
<td>Public Title of Study Modification(s)</td>
<td>clopidgorel pharmacogenetics study in normal healthy volunteers</td>
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<tr>
<td>Scientific Title of Study</td>
<td>Association of genetic polymorphisms of Cytochrome P450 2C19, ABCB1, P2Y12, Prakriti and response to clopidogrel in normal healthy participants</td>
</tr>
</tbody>
</table>

**Study procedure:**

1. Healthy normal human volunteers satisfying all the inclusion criteria and none of the exclusion criteria will be screened for the study after obtaining voluntary written informed consent. The participants will be asked to come with an overnight fast of at least 10 hours and 17 ml of blood will be collected for platelet reactivity index and biochemical, hematological and glucose tests. Further, if the platelet aggregation as assessed by platelet reactivity index is 70% participants were eligible for the enrollment. Urine samples were also collected for tests. (Sahil et al., 2015).

2. On the day of the enrollment again the participants were asked to come with an overnight fast of at least 10 hours and 9 ml of blood was collected to again check the platelet aggregation as 70% and if the platelet aggregation comes below 70%, participants were excluded from the study on the day of enrollment also. On the day of enrollment standardized breakfast was served once the samples were collected. 45 minutes following the standardized breakfast they were administered 300 mg clopidogrel and were asked to come after 4 hours with empty stomach. 5 ml and 9 ml of blood was collected after 4 hours (Brandt JT et al., 2007) as post dose respectively for genotyping and platelet reactivity index.

3. The platelet reactivity index was checked on day 7 after the drug administration and once again the participants were asked to come with an overnight fast. Day 7 (follow up) test was done so as to check the clearance of the drug from the participant’s body and the normal platelet levels which were seen prior the drug administration (Sahil et al., 2015).

Flow chart 1. Depicts the step by step procedure implemented in this study.

```
Normal healthy human volunteer
↓
17 ml blood collected for platelet reactivity index and biochemical, haematological and glucose tests. (Screening)
↓
9 ml of blood collected for platelet reactivity index.
↓
300 mg of clopidogrel administered orally.
↓
14 ml blood withdrawn for platelet reactivity index and genotyping.
↓
9 ml blood withdrawn after 7 days for platelet reactivity index.
↓
Data analysis
↓
Result and discussion
```
Platelet aggregation tests:
Platelet aggregation was studied using the turbidometric method of Born GVR. For this 9ml of blood was collected in falcon tube, containing 1ml of 3.8% sodium citrate as an anticoagulant. Platelet Rich Plasma and platelet poor plasma was obtained from this blood and Light Transmission Aggregometry was performed with 20 µM of ADP.

Genotyping:
5ml of blood was collected in 10% EDTA in falcon tube. It was centrifuged at 4 °C and the plasma discarded leaving the buffy coat. RBC and WBC left in tube were used to extract DNA by Phenol chloroform method. RBCs were lysed using a lysing solution. Then a WBC lysing solution was added, followed by addition of sodium dodecyl sulphate, proteinase kinase K and Milli Q water. This mixture was gently mixed and incubated overnight. The next morning, sodium chloride and chloroform were added, centrifuged and the supernatant was separated. Chloroform: octanol mixture was added to the supernatant and again centrifuged. To this, ice cold ethanol was added. DNA precipitates were washed with 70% ethanol. It was dried and cooled to -20°C or -80°C.

CYP2C19*2,*3, and P2Y12 genotyping:
DNA was extracted by Phenol chloroform method. Polymerase chain reaction (PCR) was carried out separately for CYP2C19*2,*3, and P2Y12 genotyping. DNA fragments were amplified with primers. Then amplified DNA was separated by horizontal gel electrophoresis containing ethidium bromide and visualized by ultraviolet Trans illumination. Restriction Fragment Length Polymorphism (RFLP) was used to genotype CYP2C19, and P2Y12.

DNA extraction:
Initial step to perform genotyping was isolation of DNA from whole blood. Isolation of DNA was carried out by Phenol-Chloroform method which had turnaround time of 2 days. For requirements, reagents and procedure refer to Annexure 3.

Conformation of isolation of DNA:
Horizontal agarose gel electrophoresis was carried out for extracted DNA samples to confirm the extraction of DNA 1% agarose gel was used to perform electrophoresis.

Polymerase Chain Reaction:
Extracted genomic DNA was amplified by Polymerase Chain Reaction using primers. PCR was performed for CYP2C19*2,*3, P2Y12 (H1 and H2 haplotype). (Annexure 4).

Restriction Fragment Length Polymorphism (RFLP):
Restriction length polymorphism (RFLP) was performed to genotype CYP2C19, and P2Y12. List of requirements, procedure and reagent preparation is given in Annexure 5.

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<th>Product size</th>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>Wild</th>
</tr>
</thead>
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<td>169 bp</td>
<td>-----------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>120 bp</td>
<td>-----------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>49 bp</td>
<td>(not seen)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product size</th>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>Wild</th>
</tr>
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<tbody>
<tr>
<td>329 bp</td>
<td>-----------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>233 bp</td>
<td>-----------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>96 bp</td>
<td>(not seen)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Product size</th>
<th>H1/H1</th>
<th>H1/H2</th>
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</thead>
<tbody>
<tr>
<td>1158</td>
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<tr>
<td>1008</td>
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<tr>
<td>645</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>580</td>
<td>-----</td>
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</tbody>
</table>

Anticipated outcome:
The study will help to assess the association between the genotypes of CYP2C19, P2Y12 polymorphisms in normal healthy human volunteers.

Statistical analysis: Test of significance:
The difference in DMPA and IPA between pre dose and 4 h post dose was tested by using Wilcoxon-signed rank sum test. Genotype frequency of CYP2C19 and P2Y12 was assessed for Hardy-Weinberg equilibrium using chi square test. Kruskal Wallis H test was used to assess the difference in DMPA and IPA between different genotypes. Association between genotypes of CYP2C19 and P2Y12 and responder status were tested using chi square test for association. A p value of <0.05 was considered as significant. All the statistical analysis was performed using GraphPad InStat version 3.05 for Windows 95.

Clopidogrel response:
Maximum platelet aggregation (MPA) was defined as the maximum percent light transmittance
that occurred during the response-monitoring period. The absolute decrease in MPA between drug-free baseline and that measured after clopidogrel administration was designated as Delta MPA (ΔMPA)

\[
\text{ΔMPA (ΔMPA)} \, (\%) = \frac{\text{Pre dose MPA} - \text{Post dose MPA}}{\text{Pre dose MPA}} \times 100
\]

Inhibition of platelet aggregation (IPA) was defined as the percent decrease in MPA from drug-free baseline and was calculated as:

\[
\text{IPA} = \frac{\text{Pre dose MPA} - \text{Post dose MPA}}{\text{Pre dose MPA}} \times 100
\]

The MPA was measured for each subject immediately before the clopidogrel loading dose. In so far as 2-4 hours are required to attain maximal platelet inhibition following a single clopidogrel loading dose. MPA measurements for this analysis were assessed at 4 hours after loading dose.

**Proposed definition of a clopidogrel poor responder:**

The response to Clopidogrel was determined on the basis of IPA. Participants having less than 30 platelet aggregation after Clopidogrel 300 mg dosage were termed as poor responders, while the participants having more than 30% platelet response were termed as normal responders. Poor responders IPA < 30 % Normal responders IPA > 30 %

**Compensation:**

The participants were paid a sum of Rs. 400/- as a token of appreciation for their participation in the study as well as to cover the costs of travel to and from the hospital.

**Determining CYP2C19 genotype:**

**Table 4. Determining CYP2C19 genotype.**

<table>
<thead>
<tr>
<th>CYP2C19*2</th>
<th>CYP2C19*3</th>
<th>CYP2C19 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>Wild</td>
<td>*1/*1</td>
</tr>
<tr>
<td>Wild</td>
<td>Hetero</td>
<td>*1/*3 (not seen)</td>
</tr>
<tr>
<td>Hetero</td>
<td>Wild</td>
<td>*1/*2</td>
</tr>
<tr>
<td>Homo</td>
<td>Wild</td>
<td>*2/*1</td>
</tr>
<tr>
<td>Homo</td>
<td>Homo</td>
<td>*2/*3 (not seen)</td>
</tr>
</tbody>
</table>

**Results**

**Demographics:**

A total of 40 adult participants were screened and 25 were enrolled. Median (range) of the age (years) was 22(18 – 31) with 11 males and 14 females. A statistically significant difference in MPA [median (range)] between pre dose [75 (65-92)] and 4 hours post dose [54(15-77)] was noted (p < 0.0001). A wide inter individual variability was noted.

**Male participants:**

In male participants (n = 11) the mean pre dose MPA was 75, the mean post dose MPA was 56.9. Median delta MPA was 22 and IPA was 30.43.

**Female participants:**

In female participants (n = 14) the mean pre dose MPA was 76.35, the mean post dose MPA was 51.57. Median delta MPA was 18 and IPA was 24.28.

**Table-5. Pre dose MPA, post dose MPA, delta MPA and IPA values [median range].**

<table>
<thead>
<tr>
<th>Participants</th>
<th>Pre dose MPA</th>
<th>Post dose MPA</th>
<th>Delta MPA</th>
<th>IPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n=11)</td>
<td>70 (65-92)</td>
<td>59 (32-77)</td>
<td>22 (2-38)</td>
<td>30.4 (2.5-54.2)</td>
</tr>
<tr>
<td>Female (n=14)</td>
<td>75.5 (70-89)</td>
<td>53.5 (15-77)</td>
<td>18 (5-59)</td>
<td>24.2 (6.6-79.7)</td>
</tr>
<tr>
<td>All (n=25)</td>
<td>75 (65-92)</td>
<td>54 (15-77)</td>
<td>18 (2-59)</td>
<td>25.7 (2.5-79.7)</td>
</tr>
</tbody>
</table>

**Genotypes and DMPA and IPA:**

**CYP2C19:**

The genotypes of CYP2C19 were found to follow Hardy – Weinberg equilibrium. Among the CYP2C19 genotypes, 14 had *1/*1, 7 had *1/*2, and 2 had *2/*2. There were no statistical significant differences in either values of DMPA or IPA between different genotypes of CYP2C19.

**P2Y12:**

The respective frequencies of P2Y12 H1 haplotype (n = 20) and P2Y12 H2 haplotype (n = 5) were 80% and 20% respectively. There is another haplotype H2/H2 which is not found yet in Indian population. No significant difference or trend was observed in DMPA or IPA values between and H2 haplotypes of P2Y12.

**Responder status and genotypes:**

Out of the 25 healthy volunteer samples, a total of 12 were normal responders and 13 were poor responders. 6 of the total 11 male volunteer samples were normal responders, remaining 5 were poor responders, 6 out of 14 female volunteer samples were normal responders, remaining 8 were poor responders. No statistically significant association was found between the genders and response to clopidogrel.
Chart 1. Comparison of male and female demographics

Chart 2. CYP2C19 Genotypes

Chart 3. P2Y₁₂ Genotypes
Figure 1. Band Pattern for CYP2C19*2

- Product Size: 169 base pair
- Well No. 1: 100bp DNA ladder
- Well No. 2, 6: Wild type
- Well no. 3, 5, 7, 8, 9: Heterozygous Mutant
- Well no. 4: Homozygous Mutant
- Restriction Enzyme: Smal
- Wild type – cuts at 120 bp & 49 bp

Figure 2. Band pattern for CYP2C19*3

- Product Size: 329 base pair
- Well No. 1: 100bp DNA ladder
- Well No. 2: Uncut
- Well No. 4, 5, 6, 8, 9, 10: Wild type
- Restriction Enzyme: BamHI
- Wild type – cuts at 233 bp & 96 bp

CYP2C19:
The maximum number of poor responders and normal responders were associated with heterozygous wild allele *1/*1, with a proportion of 46.6% and 53.3% respectively. While *2/*2 had only poor responders.

P2Y12:
The H1 haplotype of P2Y12 genotype had more number of both poor and normal responders as compared to the H2 haplotype, although no statistical significance was found.
Table 6. Cytochrome P450 2C19-MPA and IPA values [Median range].

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pre dose MPA</th>
<th>Post dose MPA</th>
<th>Delta MPA</th>
<th>IPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 *1/*1</td>
<td>75 (65-89)</td>
<td>52 (15-77)</td>
<td>22 (2-38)</td>
<td>30.4 (2.5-54.2)</td>
</tr>
<tr>
<td>(n=15)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>CYP2C19 *1/*2</td>
<td>75.5 (70-92)</td>
<td>62 (32-71)</td>
<td>18 (5-59)</td>
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<td>(n=8)</td>
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</tr>
<tr>
<td>CYP2C19 *2/*2</td>
<td>75 (70-79)</td>
<td>68 (59-77)</td>
<td>18 (2-59)</td>
<td>25.7 (2.5-79.7)</td>
</tr>
<tr>
<td>(n=2)</td>
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<td></td>
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<tr>
<td>P value (Kruskal Wallis H test)</td>
<td>0.9979</td>
<td>0.2755</td>
<td>0.1278</td>
<td>0.1406</td>
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Table 7. P2Y12 Polymorphisms: MPA and IPA values [median range].

<table>
<thead>
<tr>
<th>Genotype P2Y</th>
<th>Pre dose MPA</th>
<th>Post dose MPA</th>
<th>Delta MPA</th>
<th>IPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1/H1 (n=20)</td>
<td>76 (65-89)</td>
<td>53.5 (15-77)</td>
<td>20 (2-59)</td>
<td>28.4 (2.5-79.7)</td>
</tr>
<tr>
<td>H1/H2 (n=5)</td>
<td>72 (69-92)</td>
<td>65 (15-70)</td>
<td>5 (2-57)</td>
<td>6.6 (2.8-79.1)</td>
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<tr>
<td>P value</td>
<td>0.9413</td>
<td>0.7256</td>
<td>0.6836</td>
<td>0.6490</td>
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</table>

Table 8. Clopidogrel response.

<table>
<thead>
<tr>
<th>Clopidogrel Response</th>
<th>Male</th>
<th>Female (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR*</td>
<td>PR*</td>
<td>NR*</td>
</tr>
<tr>
<td>Participants</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

*PR = Poor Responders
*NR = Normal Responders

Figure 3. Band pattern for P2Y12.

- Product Size: 2233 base pair
- Well No. 1: 1Kb DNA ladder
- Well No. 2: Uncut
- Well No. 3: Negative control
- Well No. 4, 5, 11, 12: H1/H1
- Well No. 6, 7, 8, 9, 10, 13: H1/H2
- Restriction Enzyme: Tail
Discussions

The present study was undertaken to find out the association of genetic polymorphisms of CYP2C19 and P2Y\textsubscript{12} with phenotypic response of inhibiting platelet aggregation following a single dose of 300 mg clopidogrel in healthy adult Indians. We found a trend of decrease in platelet inhibition amongst individuals with mutant genotypes of CYP2C19. On the other hand, those with H2 haplotype (H1/H2) in P2Y\textsubscript{12} had a profound inhibition of platelet aggregation. Nearly 52% of the study participants have been found to be poor responders to clopidogrel and there was no significant difference observed between either CYP2C19 genotype or P2Y\textsubscript{12} haplotype. Clopidogrel, one of the commonly used agents in the management of thromboembolic disorders has been found to be associated with high inter-individual variability and around one-third of patients were found to be non-responsive (ER Bates et al., 2005). Amongst the various factors contributing to this variability, genetic influence of the enzymes involved in activation (CYP2C19) and metabolism (CYP3A4, CYP3A5, CYP2C9), transport (ABCB1) and pharmacodynamic target (P2Y\textsubscript{12}) are the widely studied one. Of these, the present study had assessed the influence of CYP2C19 and P2Y\textsubscript{12} polymorphisms with their effect on the degree of platelet inhibition. The allele frequencies of *2 and *3 of CYP2C19 in Asian population have been found to be range between 29-35% and 2-9% respectively.
The following images are achieved using SPSS v20 software:

(Jose R et al., 2005). Depending on the activity, those with *1/*1 genotype in CYP2C19 were termed as extensive metabolizers, *1/*2 as intermediate and the rest (*1/*3, *2/*2, *2/*3 and *3/*3) as poor metabolizers (Dean L et al., 2012). We found majority of the individuals with *1/*1 genotype (60%) followed by *1/*2 (32%) contrasting to other studies from the Indian population (T S Panchabai et al., 2006).

Although statistically not significant, a trend was observed in decreased response to clopidogrel with mutant genotypes as compared to wild although several other authors have established a significant association either in healthy volunteers or patients (T S Panchabai et al., 2006) and (Giusti B et al., 2009). A similar trend was also observed in another study with drug-naive patients of acute myocardial
infarction from the same population (Shaila K K et al., 2013). This can be either attributed to influence of other genetic factors that were not assessed or an inadequate sample size in the present study. Also, because of the aforementioned reason, we did not find any significant difference between carriers and non-carriers of reduced function alleles. Interestingly, Mega and her coworkers (Mega J L et al., 2009) has found that carriers of at least one of these reduced function alleles had a relative reduction of 32.4% of the active metabolite of clopidogrel in their plasma as compared to non-carriers. Additionally, the authors in the same study have also found a 9% decrease in the maximum platelet aggregation amongst carriers of any of these reduced function alleles. Even United States food and drug administration (USFDA) has recently revised the prescribing information highlighting the impact of CYP2C19 genotyping with clopidogrel response (Ellis K J et al., 2009). US FDA has even issued a "black-box" warning of reduced effectiveness of clopidogrel in patients with *2 alleles and recommends either initiation of clopidogrel at higher dose or other alternative anti-platelet agent (Holmes D R et al., 2010). Considering the prevalence of mutant alleles of CYP2C19 in Indian population, it may be worthwhile to do genetic testing to determine the adequate dose of clopidogrel but there is a paucity of data on the cost-effectiveness of this strategy. Studies evaluating the cost-effectiveness of performing pharmacogenetic testing for clopidogrel administration are the need of the hour to determine their clinical utility especially in Indian set-up. We also found a decrease in inhibition of platelet aggregation with H2 haplotype of P2Y12 which is contrasting to earlier studies (Malek LA et al., 2008). Interestingly, H2 haplotype has been found to be a high risk factor for coronary artery disease (Cavallari U et al., 2007) and peripheral arterial disease (Fontana P et al., 2003). Due to an increased ADP induced platelet aggregation. Although combination of H2 haplotype with CYP2C19 mutant genotype has been shown to have altered anti-platelet response following clopidogrel response, (Malek LA et al., 2008) and (Shaila K K et al., 2013) we had only two patients in the present study leaving no such conclusions.

Conclusion

A dose of 300 mg was found to be safe in all our healthy participants as no adverse dose of events occurred during the course of our study. Approximately 13 of the volunteers were found to be poor responder to 300mg of clopidogrel suggesting that clopidogrel needs to be administered in larger dose. Some of the previous studies have suggested increasing the dose of clopidogrel to 600mg in poor responders. No statistically significant association was noted between both genotypes (CYP2C19, P2Y12) and inhibition of platelet aggregation in this study of small sample size. The use of Clopidogrel is increasing, so is the incidence of ACS. As of now it is too early to say that laboratory investigations of every person receiving clopidogrel should be done. But larger studies should be done in Indian population, in order to identify and classify the response to Clopidogrel and its association to genetic polymorphisms. This will lead to better therapeutics and lower the rate of secondary events, ultimately making Clopidogrel a better and safer drug.

Limitations of study:

This study was conducted on a very small sample size of healthy volunteers. Also the platelet aggregation tests to quantify Clopidogrel response were done by LTA, which has many variables that can cloud the results. This may be one of the reasons we were not able to find out any significance between the various polymorphisms and Clopidogrel response. Other co-morbid conditions and concomitant diseases may yet present a different kind of picture. Also we did not measure the plasma concentration of the active metabolite of Clopidogrel. The study results should be interpreted with the limitations of not having assessed the influence of other genes involved in the pharmacokinetics of clopidogrel; additionally, in CYP2C19 alleles, presence of *17 (has ultra-rapid activity) was not looked at; patients on clopidogrel have not been included and therefore data on clinical endpoints were not available.

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Conflict of Interests:

Authors declare that there is no conflict of interests regarding the publication of this paper.
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